

## **Detection of CD45 (LCA) in Formalin-Fixed, Paraffin-Embedded Mouse Tissue**

### **Reagent and Antibody Information**

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Primary Antibody: Rat Monoclonal CD45 Antibody (Biotin)

GeneTex, Inc

San Antonio, TX 78245

[www.genetex.com](http://www.genetex.com)

1-877-436-3839

Catalog # GTX19592

Label Complex: Peroxidase-Conjugated Streptavidin SS Label

Biogenex Laboratories

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Catalog # HK330-9K

## **Staining Procedure**

Positive Control Tissue: Thymus and spleen - hematopoietic cells (except erythrocytes)

Stain Localization: Cell membrane

1. Deparaffinize and hydrate slides through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* \_\_\_\_\_

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

6. Avidin / Biotin Blocking Kit

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X Wash Buffer.

Apply biotin block for 15 minutes at room temperature.

**DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BUFFER.**

7. Apply primary antibody at a 1:250 dilution. Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

8. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

9. Apply the Streptavidin SS Label. Incubate for 30 minutes at room temperature.

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

10. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

11. Apply the DAB chromagen. Incubate in the dark for 6 minutes at room temperature.  
(Add 1 drop of DAB per ml of substrate)

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

12. Rinse the slides in tap water 3 minutes.

13. Counterstain with Harris Hematoxylin for 20 seconds.

14. Rinse the slides in tap water until water is clear.

15. Gently agitate slides in 1X Wash Buffer until the tissues turn blue.

16. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

17. Coverslip

*Updated 06/13/07*